

Set	Items	Description
S1	43	AU="BENNETT D.C."
S2	4	AU="CAUCHON E"
S3	1367	E3-E41
S4	18	E3-E11
S5	7	E3-E4
S6	6	AU=DANAGHER P
S7	7	E3-E4
S8	1107	E3-E50
S9	827	E1-E21
S10	3360	S1-S9
S11	2276	HEPARINASE?
S12	508	HEPARANASE?
S13	2751	S11 OR S12
S14	5	S13 AND S10
S15	4	RD (unique items)
S16	627906	INFLAMMAT?
S17	123	S16 AND S13

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(FILE 'USPAT' ENTERED AT 16:29:17 ON 22 SEP 1997)

	E BENNETT, D. CLARK/IN
	E CAUCHON, ELIZABETH/IN
	E FINK, DOMINIQUE/IN
	E GROUX, BRIGETTE/IN
	E DANAGHER, PAMELA/IN
	E ZIMMERMAN, JOSEPH/IN
L1	13 S E3-E7
L2	117 S HEPARINASE?
L3	16760 S INFLAMMATORY
L4	19 S L3 AND L2
	E HSIA, ARIANE/IN
	E DANAGHER, PAMELA/IN

Order
PATENTS

(FILE 'USPAT' ENTERED AT 16:29:17 ON 22 SEP 1997)

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E CAUCHON, ELIZABETH/IN
E FINK, DOMINIQUE/IN
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L1 13 S E3-E7
L2 117 S HEPARINASE?
L3 16760 S INFLAMMATORY
L4 19 S L3 AND L2

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DANAGHER

> t cit ab 3

3. 5,169,772, Dec. 8, 1992, Large scale method for purification of high purity heparinase from flavobacterium heparinum; **Joseph J. Zimmerman**, et al., 435/232, 252.1, 850 :IMAGE AVAILABLE:

US PAT NO: 5,169,772 :IMAGE AVAILABLE:

L1: 3 of 13

ABSTRACT:

The present invention is an improved process for purification of active heparinase and heparinase like enzymes from Gram negative organisms, in particular, Flavobacterium heparinum. The primary advantage of the process is the fact that it allows large scale processing and high yield of heparinase. The heparinase is released from the periplasmic space of the organism by osmotic shock treatment, first into an osmotically stabilized medium, secondly into a non-stabilized medium having a pH of approximately pH 6.0 and 8.6 with subsequent release into a second non-stabilized medium containing approximately 0.15 M sodium chloride, followed by fractionation by cation exchange chromatography, and, optionally, electrophoresis or gel filtration chromatography. Two proteins having heparinase activity have been isolated, one having a molecular weight of approximately 42,000 Daltons and the other having a molecular weight of 65,000 to 75,000 Daltons. Also described is the construction of a library for screening for the genes encoding the proteins having heparinase activity and two assay for detecting organisms producing heparinase, either F. heparinum or genetically engineered organisms.

heparanase

=> t cit 1-19

1. 5,652,014, Jul. 29, 1997, Medicament coated refractive anterior chamber ocular implant; Miles A. Galin, et al., 427/2.24, 2.1, 536; 623/6, 901 :IMAGE AVAILABLE:
2. 5,630,978, May 20, 1997, Preparation of biologically active molecules by molecular imprinting; Abraham J. Domb, 264/330, 331.11, 331.16, 331.19; 424/78.08, 78.37; 526/238.1, 238.2 :IMAGE AVAILABLE:
3. 5,627,265, May 6, 1997, Receptor for cell-binding domain of thrombospondins; William A. Frazier, et al., 530/350, 395 :IMAGE AVAILABLE:
4. 5,618,710, Apr. 8, 1997, Crosslinked enzyme crystals; Manuel A. Navia, et al., 435/174; 424/94.1, 94.6, 94.63; 435/41, 109, 195, 198, 212, 218, 817; 436/518; 530/413, 810 :IMAGE AVAILABLE:
5. 5,583,121, Dec. 10, 1996, Non-anticoagulant chemically modified heparinoids for treating hypovolemic shock and related shock syndromes; Irshad H. Chaudry, et al., 514/56, 921; 536/21 :IMAGE AVAILABLE:
6. 5,571,506, Nov. 5, 1996, Aromatic oligomeric compounds useful as mimics of bioactive macromolecules; John R. Regan, et al., 424/78.17, 78.37; 514/822, 824; 528/139, 141, 143, 148, 149, 150, 151 :IMAGE AVAILABLE:
7. 5,567,417, Oct. 22, 1996, Method for inhibiting angiogenesis using **heparinase**; Ramnath Sasisekharan, et al., 424/94.5; 435/232 :IMAGE AVAILABLE:
8. 5,552,267, Sep. 3, 1996, Solution for prolonged organ preservation; David M. Stern, et al., 435/1.1, 1.2 :IMAGE AVAILABLE:
9. 5,541,166, Jul. 30, 1996, Sulphated polysaccharides having anti-metastatic and/or anti-inflammatory activity; Christopher R. Parish, et al., 514/56, 54, 59; 536/21, 53, 54, 55, 55.1, 55.3 :IMAGE AVAILABLE:
10. 5,474,987, Dec. 12, 1995, Methods of using low molecular weight heparins treatment of pathological processes; Irun R. Cohen, et al., 514/56, 54, 825; 536/21, 54, 55 :IMAGE AVAILABLE:
11. 5,459,068, Oct. 17, 1995, Microassay system for assessing transmigration of PMN across epithelia in a serosal-to-mucosal direction; James L. Madara, 435/287.1, 287.2, 287.9, 288.1 :IMAGE AVAILABLE:
12. 5,362,641, Nov. 8, 1994, Heparanase derived from human Sk-Hep-1 cell line; Zvi Fuks, et al., 435/209, 195, 200, 201 :IMAGE AVAILABLE:
13. 5,302,384, Apr. 12, 1994, Endothelial-derived IL-8 Adhesion Inhibitor; Michael A. Gimbrone, Jr., et al., 424/85.2; 514/21; 530/351 :IMAGE AVAILABLE:
14. 5,013,724, May 7, 1991, Process for the sulfation of glycosaminoglycans, the sulfated glycosaminoglycans and their biological applications; Maurice Petitou, et al., 514/54, 53, 56, 61, 885; 536/21, 54, 55.2, 55.3, 117, 122, 123, 124 :IMAGE AVAILABLE:

What is claimed is

1. A method to decrease localized inflammatory responses arising from an ischemia/reperfusion injury in a tissue of a patient comprising intravascularly administering to said patient heparinase enzyme in an effective amount sufficient to decrease neutrophil transmigration through activated endothelium and basement membrane of said vasculature which decreases said localized inflammatory response arising from an ischemia/reperfusion injury.
2. The method of claim 1, wherein said administration of said heparinase enzyme removes and digests heparin and heparan sulfate from endothelial cell surfaces and extracellular matrices of said tissue.
3. The method of claim 1, wherein said administration of said heparinase enzyme decreases the accumulation of leukocytes in tissue adjacent to endothelial cell surfaces and extracellular matrices.
4. The method of claim 1, wherein said administration of said heparinase enzyme inhibits leukocyte extravasation by releasing immobilized chemokines from the endothelium.
5. The method of claim 1, wherein said administration of said heparinase enzyme inhibits leukocyte rolling on endothelium.
6. The method of claim 1, wherein said heparinase enzyme is expressed from a recombinant nucleotide sequence, in *Flavobacterium heparinum*.
7. The method of claim 1, wherein said heparinase enzyme is expressed from a recombinant nucleotide sequence in an organism in which it does not naturally occur.
18. The method of claim 1, wherein said heparinase enzyme is heparinase III.
19. The method of claim 1, wherein said ischemia/reperfusion injury is selected from the group consisting of myocardial infarction, stroke, organ transplant, traumatic shock, cardiovascular surgery.

15. 5,001,116, Mar. 19, 1991, Inhibition of angiogenesis; Moses J. Folkman, et al., 514/56, 171, 177, 178, 182 :IMAGE AVAILABLE:
16. 4,994,443, Feb. 19, 1991, Inhibition of angiogenesis; Moses J. Folkman, et al., 514/56, 177, 178 :IMAGE AVAILABLE:
17. 4,981,955, Jan. 1, 1991, Depolymerization method of heparin; Lorenzo L. Lopez, 536/21, 18.7, 55.3, 121 :IMAGE AVAILABLE:
18. 4,731,080, Mar. 15, 1988, Coated intraocular lens; Miles A. Galin, 623/6; 427/2.24; 623/66 :IMAGE AVAILABLE:
19. 4,240,163, Dec. 23, 1980, Medicament coated intraocular lens; Miles A. Galin, 623/6; 427/2.24 :IMAGE AVAILABLE:

Set	Items	Description
S1	43	AU="BENNETT D.C."
S2	4	AU="CAUCHON E"
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S10	3360	S1-S9
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S12	508	HEPARANASE?
S13	2751	S11 OR S12
S14	5	S13 AND S10
S15	4	RD (unique items)
S16	627906	INFLAMMAT?
S17	123	S16 AND S13
S18	62	RD (unique items)

t s18/3,ab/ 3, 4, 11, 12,15,18, 19, 20, 22, 42, 43, 46, 52, 58, 59

18/3,AB/3 (Item 3 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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08740123 95052656

Regulation of adhesion of CD4+ T lymphocytes to intact or **heparinase** -treated subendothelial extracellular matrix by diffusible or anchored RANTES and MIP-1 beta.

Gilat D; Hershkovich R; Mekori YA; Vlodavsky I; Lider O

Department of Cell Biology, Weizmann Institute of Science, Rehovot, Israel.

J Immunol (UNITED STATES) Dec 1 1994, 153 (11) p4899-906, ISSN 0022-1767 Journal Code: IFB

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Chemokines, a superfamily of 8- to 11-kDa mediators of **inflammation**, affect the attachment of immune cells to vascular endothelia by binding to cell surface glycosaminoglycans. We analyzed whether chemokines are also involved in interactions between CD4+ T lymphocytes and the subendothelial extracellular matrix (ECM). Soluble mediators, such as MIP-1 beta and RANTES, induced the binding of resting human CD4+ T cells to ECM in an integrin-dependent manner. Both MIP-1 beta and RANTES bound to intact ECM and retained their adhesive properties, and moreover, ECM-bound RANTES and MIP-1 beta prolonged the time course of interactions between the CD4+ T cells and the ECM. Because the adhesive effect of these chemokines was restricted by an inhibitor of GTP-binding proteins, the adhesive effect of ECM-bound RANTES and MIP-1 beta, which requires an intact cytoskeleton, seems to involve activation of a G protein-linked receptor. MIP-1 beta and RANTES exert their pro-adhesive effects through interactions with glycosaminoglycans, because **heparinase** -treated ECM did not bound chemokines and because the chemokines ability to induce T cell adhesion was abrogated if: 1) either of the chemokines is pretreated with heparin or heparan-sulfate (HS), 2) HS is removed from intact ECM by **heparinase**, an HS-specific endoglycosidase, or 3) the ECM-bound chemokines are released by pretreatment with **heparinase**. Hence, the adhesive effects of immobilized chemokines is not restricted to T cells interacting with endothelial cells, but also affects the migration of immune cells which reside and function in the context of ECM.

18/3,AB/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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08719654 96413763

Shedding of heparan sulfate proteoglycan by stimulated endothelial cells: evidence for proteolysis of cell-surface molecules.

Ihrcke NS; Platt JL

Department of Surgery, Duke University Medical Center Durham, North Carolina 27710, USA.

J Cell Physiol (UNITED STATES) Sep 1996, 168 (3) p625-37, ISSN 0021-9541 Journal Code: HNB

Contract/Grant No.: HL46810, HL, NHLBI; DK38108, DK, NIDDK; HL50985, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Activation of endothelial cells by cytokines and endotoxin causes procoagulant and pro-inflammatory changes over a period of hours. We postulated that the same functional state might be achieved more rapidly by changes in the metabolism of heparan sulfate, which supports many of the normal functions of endothelial cells. We previously found that binding of anti-endothelial cell antibodies and activation of complement on endothelial cells causes the rapid shedding of endothelial cell heparan sulfate. Here we report the biochemical mechanism responsible for the release of the heparan sulfate. Stimulation of endothelial cells by anti-endothelial cell antibodies and complement resulted in the release of 35S-heparan sulfate proteoglycan and partially degraded 35S-heparan sulfate chains. Degradation of the 35S-heparan sulfate chains was not necessary for release since heparin and suramin prevented cleavage of the heparan sulfate but did not inhibit release from stimulated endothelial cells. The 35S-heparan sulfate proteoglycan released from endothelial cells originated from the cell surface and had a core protein similar in size (70.5 kD) to syndecan-1. Release was due to proteolytic cleavage of the protein core by serine and/or cysteine proteinases since the release of heparan sulfate was inhibited 87% by antipain and 53% by leupeptin. Release of heparan sulfate coincided with a decrease of approximately 7 kD in the mass of the protein core and with a loss of hydrophobicity of the proteoglycan, consistent with the loss of the hydrophobic transmembrane domain. The cleavage and release of cell-surface 35S-heparan sulfate proteoglycan might be a novel mechanism by which endothelial cells may rapidly acquire the functional properties of activated endothelial cells.

18/3,AB/11 (Item 11 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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08265413 95281591

A disaccharide that inhibits tumor necrosis factor alpha is formed from the extracellular matrix by the enzyme **heparanase**.

Lider O; Cahalon L; Gilat D; Hershkovich R; Siegel D; Margalit R; Shoseyov O; Cohen IR

Department of Cell Biology, Weizmann Institute of Science, Rehovot, Israel.

Proc Natl Acad Sci U S A (UNITED STATES) May 23 1995, 92 (11) p5037-41
, ISSN 0027-8424 Journal Code: PV3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The activation of T cells by antigens or mitogens leads to the secretion of cytokines and enzymes that shape the **inflammatory** response. Among these molecular mediators of **inflammation** is a **heparanase** enzyme that degrades the heparan sulfate scaffold of the extracellular matrix (ECM). Activated T cells use **heparanase** to penetrate the ECM and gain access to the tissues. We now report that among the breakdown products of the ECM generated by **heparanase** is a trisulfated disaccharide that can inhibit delayed-type hypersensitivity (DTH) in mice. This inhibition of T-cell mediated **inflammation** in vivo was associated with an inhibitory effect of the disaccharide on the production of biologically active tumor necrosis factor alpha (TNF-alpha) by activated T cells in vitro; the trisulfated disaccharide did not affect T-cell viability or responsiveness generally. Both the in vivo and in vitro effects of the disaccharide manifested a bell-shaped dose-response curve. The inhibitory effects of the trisulfated disaccharide were lost if the sulfate groups were removed. Thus, the disaccharide, which may be a natural product of **inflammation**, can regulate the functional nature of the response by the T cell to activation. Such a feedback control mechanism could enable the T cell to assess the extent of tissue degradation and adjust its behavior accordingly.

18/3,AB/12 (Item 12 from file: 155)

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08228963 95239139

Molecular behavior adapts to context: **heparanase** functions as an extracellular matrix-degrading enzyme or as a T cell adhesion molecule, depending on the local pH.

Gilat D; HersHKoviz R; Goldkorn I; Cahalon L; Korner G; Vlodavsky I; Lider O

Department of Cell Biology, Weizmann Institute of Science, Rehovot, Israel.

J Exp Med (UNITED STATES) May 1 1995, 181 (5) p1929-34, ISSN 0022-1007 Journal Code: I2V

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Migration of lymphocytes into **inflammatory** sites requires their adhesion to the vascular endothelium and subendothelial extracellular matrix (ECM). The ensuing penetration of the ECM is associated with the expression of ECM-degrading enzymes, such as endo-beta-D glucuronidase (**heparanase**), which cleaves heparan sulfate (HS) proteoglycans. We now report that, depending on the local pH, a mammalian **heparanase** can function either as an enzyme or as an adhesion molecule. At relatively acidified pH conditions, **heparanase** performs as an enzyme, degrading HS. In contrast, at the hydrogen ion concentration of a quiescent tissue, **heparanase** binds specifically to HS molecules without degrading them, and thereby anchors CD4+ human T lymphocytes. Thus, the local state of a tissue can regulate the activities of **heparanase** and can determine whether the molecule will function as an enzyme or as a proadhesive molecule. or 2

18/3,AB/15 (Item 15 from file: 155)

08157528 95155420

CXC chemokines connective tissue activating peptide-III and neutrophil activating peptide-2 are heparin/heparan sulfate-degrading enzymes.

Hoogewerf AJ; Leone JW; Reardon IM; Howe WJ; Asa D; Heinrikson RL; Ledbetter SR

Units of Cancer & Infectious Disease, Upjohn Company, Kalamazoo, Michigan 49001.

J Biol Chem (UNITED STATES) Feb 17 1995, 270 (7) p3268-77, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Heparan sulfate proteoglycans at cell surfaces or in extracellular matrices bind diverse molecules, including growth factors and cytokines, and it is believed that the activities of these molecules may be regulated by the metabolism of heparan sulfate. In this study, purification of a heparan sulfate-degrading enzyme from human platelets led to the discovery that the enzymatic activity resides in at least two members of the platelet basic protein (PBP) family known as connective tissue activating peptide-III (CTAP-III) and neutrophil activating peptide-2. PBP and its N-truncated derivatives, CTAP-III and neutrophil activating peptide-2, are CXC chemokines, a group of molecules involved in **inflammation** and wound healing. SDS-polyacrylamide gel electrophoresis analysis of the purified **heparanase** resulted in a single broad band at 8-10 kDa, the known molecular weight of PBP and its truncated derivatives. Gel filtration chromatography of **heparanase** resulted in peaks of activity corresponding to monomers, dimers, and tetramers; these higher order aggregates are known to form among the chemokines. N-terminal sequence analysis of the same preparation indicated that only PBP and truncated derivatives were present, and commercial CTAP-III from three suppliers had **heparanase** activity. Antisera produced in animals immunized with a um

C-terminal synthetic peptide of PBP inhibited **heparanase** activity by 95%, compared with activity of the purified enzyme in the presence of the preimmune sera. The synthetic peptide also inhibited **heparanase** by 95% at 250 microM, compared to the 33% inhibition of **heparanase** activity by two other peptides. The enzyme was determined to be an endoglucosaminidase, and it degraded both heparin and heparan sulfate with optimal activity at pH 5.8. Chromatofocusing of the purified **heparanase** resulted in two protein peaks: an inactive peak at pI 7.3; and an active peak at pI 4.8-5.1. Sequence analysis showed that the two peaks contained identical protein, suggesting that a post-translational modification activates the enzyme.

18/3,AB/18 (Item 18 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07237930 93014709

Lymphocyte migration through extracellular matrix.
Ratner S
Breast Cancer Program, Meyer L. Prentis Comprehensive Cancer Center of
Metropolitan Detroit, Mich.
Invasion Metastasis (SWITZERLAND) 1992, 12 (2) p82-100, ISSN
0251-1789 Journal Code: GV4
Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC
The movement of lymphocytes through extracellular matrix (ECM) is an essential component of normal traffic and infiltration into **inflammatory** sites. This review surveys current knowledge of the mechanisms of lymphocyte migration through ECM, most of which was derived from work with in vitro models of basement membranes, interstitial stroma, or their constituent components. Normal lymphocyte motility is an extremely plastic property. Naive lymphocytes tend to be unresponsive to ECM components and many chemoattractants, but when exposed to antigens, artificial mitogens and certain lymphokines, they rapidly acquire locomotory capacity, which is expressed as increased polarity, adhesiveness, invasiveness and chemotactic response. Acquisition of locomotory capacity is associated with the G0/G1 transition, and activation of protein kinase C appears to be a key event. Preliminary evidence indicates that mitogenesis and differentiation to the memory phenotype trigger a long-lasting, possibly permanent elevation of locomotory response to ECM. Receptors for fibronectin, laminin and collagens I and IV have been implicated as mediators of lymphocyte motility, but these receptors have not been characterized in detail. **Heparanases** facilitate T cell movement through the basement membrane, but the role of proteases has not yet been defined. Major gaps remain in our understanding of the connection between in vitro models and specific stages of the infiltration process in vivo and of motility regulation at the molecular level.

18/3,AB/19 (Item 19 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07237927 93014705

Expression of **heparanase** by platelets and circulating cells of the immune system: possible involvement in diapedesis and extravasation.
Vlodavsky I; Eldor A; Haimovitz-Friedman A; Matzner Y; Ishai-Michaeli R; Lider O; Naparstek Y; Cohen IR; Fuks Z
Department of Oncology, Hadassah University Hospital, Jerusalem, Israel.
Invasion Metastasis (SWITZERLAND) 1992, 12 (2) p112-27, ISSN
0251-1789 Journal Code: GV4
Contract/Grant No.: CA-30289, CA, NCI; CA-52462, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC

Interaction of T and B lymphocytes, platelets, granulocytes, macrophages and mast cells with the subendothelial extracellular matrix (ECM) is associated with degradation of heparan sulfate (HS) by a specific endoglycosidase (**heparanase**) activity. The enzyme is released from intracellular compartments (i.e., lysosomes, specific granules) in response to various activation signals (i.e., thrombin, calcium ionophore, immune complexes, antigens, mitogens), suggesting its regulated involvement in **inflammation** and cellular immunity. In contrast, various tumor cells appear to express and secrete **heparanase** in a constitutive manner, in correlation with their metastatic potential. **Heparanase** enzymes produced by different cell types may exhibit different molecular properties and substrate cleavage specificities. The platelet enzyme appears also in a latent form. It can be activated by tumor cells and thereby facilitate their extravasation in the process of metastasis. Degradation of ECM-HS by all cell types was facilitated by a proteolytic activity residing in the ECM and/or expressed by the invading cells. This proteolytic activity produced a more accessible substrate for the **heparanase** enzymes. **Heparanase**-inhibiting, nonanticoagulant species of heparin markedly reduced the incidence of lung metastasis in experimental animals. These species of heparin also significantly impaired the traffic of T lymphocytes and suppressed cellular immune reactivity and experimental autoimmune diseases. **Heparanase** activity expressed by intact cells (i.e., platelets, mast cells, neutrophils, lymphoma cells) was found to release active HS-bound basic fibroblast growth factor from ECM and basement membranes. **Heparanase** may thus elicit an indirect neovascular response in processes such as wound repair, **inflammation** and tumor development. The significant anticancerous effect of **heparanase**-inhibiting molecules may therefore be attributed to their potential inhibition of both tumor invasion and angiogenesis. Both normal leukocytic cells and metastatic tumor cells can enter the bloodstream, travel to distant sites and extravasate to the parenchyma at these sites. We suggest that **heparanase** is utilized for this purpose by both types of cells. Other functions (i.e., enzyme activities, adhesive interactions, chemotactic and proliferative responses) of metastatic tumor cells seem to mimic the equivalent functions of leukocytes as they migrate across blood vessels to gain access to sites of **inflammation**.

18/3,AB/20 (Item 20 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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07153134 92307041

Thrombin enhances degradation of heparan sulfate in the extracellular matrix by tumor cell **heparanase**.

Benezra M; Vlodavsky I; Bar-Shavit R

Department of Oncology, Hadassah University Hospital, Jerusalem, Israel.

Exp Cell Res (UNITED STATES) Jul 1992, 201 (1) p208-15, ISSN 0014-4827 Journal Code: EPB

Contract/Grant No.: CA-30289, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The ability of normal and malignant blood-borne cells to extravasate correlates with the activity of an endo-beta-D-glucuronidase (**heparanase**) which degrades heparan sulfate (HS) in the subendothelial extracellular matrix (ECM). The association of malignancy with different types of coagulopathies prompted us to study the effect of thrombin (EC 3.4.21.5), a serine protease elaborated during activation of the clotting cascade, on the ability of **heparanase** to degrade the ECM-HS. The circulating zymogen form of thrombin, prothrombin, was converted to proteolytically active thrombin during incubation with ECM. Thrombin generation by the ECM was time and dose dependent, reaching maximal conversion by 6 h incubation at 3 U/ml of prothrombin. **Heparanase**-mediated release of low Mr HS cleavage products from sulfate-labeled ECM was stimulated four- to sixfold in the presence of alpha-thrombin, but

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there was no effect on degradation of soluble HS. Similar results were obtained with **heparanase** preparations derived from mouse lymphoma and human hepatoma cell lines and from human placenta. Incubation of ECM with alpha-thrombin alone resulted in release of nearly intact high-Mr labeled proteoglycans. Thrombin stimulation of **heparanase** action was dose and time dependent, reaching a maximal value at 24 h incubation with 1 microm alpha-thrombin. The effect of modified thrombin preparations correlated with their proteolytic activity. Catalytically blocked preparations of thrombin (e.g., DIP-alpha-thrombin, MeSO2-alpha-thrombin) failed to facilitate **heparanase** action, while catalytically modified preparations (e.g., gamma-thrombin, NO2-alpha-thrombin) exerted only a slight enhancement. Antithrombin III (ATIII) and hirudin both inhibited thrombin-stimulated **heparanase** degradation of ECM-bound HS. **Heparanase** action was also facilitated by ECM-immobilized thrombin to an extent which was similar to that induced by soluble thrombin. This result implies that thrombin sequestered by the subendothelial ECM and protected from interaction with its natural inhibitor ATIII (Bar-Shavit et al., 1989, J. Clin. Invest. 84, 1096-1104) may participate locally in cellular invasion during tumor metastasis, **inflammation**, and autoimmunity.

18/3,AB/22 (Item 22 from file: 155)
DIALOG(R) File 155:MEDLINE(R)
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06581321 91208223

Heparanase activity expressed by platelets, neutrophils, and lymphoma cells releases active fibroblast growth factor from extracellular matrix.

Ishai-Michaeli R; Eldor A; Vlodavsky I
Department of Oncology, Hadassah, Hebrew University Hospital, Jerusalem, Israel.

Cell Regul (UNITED STATES) Oct 1990, 1 (11) p833-42, ISSN 1044-2030
Journal Code: A1U

Contract/Grant No.: CA30289, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Incubation of platelets, neutrophils, and lymphoma cells with Descemet's membranes of bovine corneas and with the extracellular matrix (ECM) produced by cultured corneal endothelial cells resulted in release of basic fibroblast growth factor (bFGF), which stimulated the proliferation of 3T3 fibroblasts and vascular endothelial cells. Similar requirements were observed for release of endogenous bFGF stored in Descemet's membrane and of exogenous bFGF sequestered by the subendothelial ECM. Release of ECM-resident bFGF by platelets, neutrophils, and lymphoma cells was inhibited by carrageenan lambda, but not by protease inhibitors, in correlation with the inhibition of **heparanase** activity expressed by these cells. Degradation of the ECM-heparan sulfate side chains by this endo-beta-D-glucuronidase is thought to play an important role in cell invasion, particularly in the extravasation of blood-borne tumor cells and activated cells of the immune system. We propose that both **heparanase** and ECM-resident bFGF may modulate the cell response to contact with its local environment. **Heparanase**-mediated release of active bFGF from storage in basement membranes provides a novel mechanism for a localized induction of neovascularization in various normal and pathological processes, such as wound healing, **inflammation**, and tumor development.

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18/3,AB/42 (Item 1 from file: 73)
DIALOG(R) File 73:EMBASE
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10081864 EMBASE No: 96239924

Interactions of migrating T lymphocytes, **inflammatory** mediators, and the extracellular matrix

Lider O.; HersHKoviz R.; Kachalsky S.G.

Department of Immunology, Weizmann Institute of Science, P. O. Box 26, Rehovot 76100 Israel

Critical Reviews in Immunology (USA) , 1995, 15/3-4 (271-283) CODEN: CCRID ISSN: 1040-8401

LANGUAGES: English SUMMARY LANGUAGES: English

Leukocytes are mobile units of the immune system. The process of leukocytes migration from blood vessels to inflamed tissues involves two major steps: (1) extravasation through the vessel wall and (2) movement through the underlying basement membrane and extracellular matrix (ECM). The ECM is a complex macromolecular mesh composed of proteoglycans and adhesive glycoproteins, such as collagen, laminin, and fibronectin, and serves as a supportive structure surrounding cells and can also provide co-stimulatory signals to immune cells. Hence, the basement membrane and the ECM play important roles as contexts in which biological processes take place, and therefore these moieties should be considered as microenvironment milieu in which extravasating cells function, communicate, and signal their messages; the outcome of which can result in the immunological eradication of hazardous elements. During migration, leukocytes continuously exchange information with the surrounding microenvironment. This cross-talk, which is also influenced by cytokines and chemokines, determines the type and the strength of the resulting immune response to foreign determinants. As suggested in the present article, these signals determine the response to a specific antigen and enable the migrating leukocytes to recognize any insult in their vicinity and to rapidly modify their activities.

18/3,AB/43 (Item 2 from file: 73)

DIALOG(R)File 73:EMBASE

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9839630 EMBASE No: 96007485

Interplay of T cells and cytokines in the context of enzymatically modified extracellular matrix

Gilat D.; Cahalon L.; HersHKoviz R.; Lider O.

Department of Cell Biology, Weizmann Institute of Science, Rehovot 76100 Israel

Immunology Today (United Kingdom) , 1996, 17/1 (16-20) CODEN: IMTOD ISSN: 0167-5699

LANGUAGES: English SUMMARY LANGUAGES: English

Following immunological insult, T cells migrate from blood vessels to **inflammatory** sites through the extracellular matrix (ECM). This movement is regulated by signals provided by proinflammatory mediators, including cytokines, chemokines and ECM-degrading enzymes. Here, Dalia Gilat and colleagues discuss the interactions between tissue-invading T cells and locally secreted, diffusible or ECM-anchored mediators.

18/3,AB/46 (Item 1 from file: 5)

DIALOG(R)File 5:BIOSIS PREVIEWS(R)

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13483572 BIOSIS Number: 99483572

Heparinase treatment decreases neutrophil-specific interactions with inflamed endothelial cells in vitro

Bennett D C; Grouix B; Cauchon E

IBEX Technologies, Montreal, PQ H4P 1P7, Canada

FASEB Journal 11 (3). ~~1997. A116.~~

Full Journal Title: Annual Meeting of the Professional Research Scientists on Experimental Biology 97, New Orleans, Louisiana, USA, April 6-9, 1997. FASEB Journal

ISSN: 0892-6638

5/3,AB/1 (Item 1 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci
(c) 1997 Inst for Sci Info. All rts. reserv.

15494451 Genuine Article#: WL530 Number of References: 0
Title: **Heparinase** treatment decreases neutrophil-specific interactions with inflamed endothelial cells in vitro.
Author(s): Bennett DC; Grouix B; **Cauchon E**
Corporate Source: IBEX TECHNOL./MONTREAL/PQ H4P 1P7/CANADA/
Journal: FASEB JOURNAL, 1997, V11, N3 (FEB 28), P681-681
ISSN: 0892-6638 Publication date: 19970228
Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998
Language: English Document Type: MEETING ABSTRACT

15/3,AB/2 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 1997 Elsevier Science B.V. All rts. reserv.

10045196 EMBASE No: 96240862
Isolation and expression in Escherichia coli of hepB and hepC, genes coding for the glycosaminoglycan-degrading enzymes **heparinase** II and **heparinase** III, respectively, from Flavobacterium heparinum
Su H.; Blain F.; Musil R.A.; Zimmermann J.J.F.; Gu K.; **Bennett D.C.**
IBEX Technologies, 5485 Pare, Montreal, Que. H4P 1P7 Canada
Applied and Environmental Microbiology (USA) , 1996, 62/8 (2723-2734)
CODEN: AEMID ISSN: 0099-2240
LANGUAGES: English SUMMARY LANGUAGES: English
Upon induction with heparin, Flavobacterium heparinum synthesizes and secretes into its periplasmic space **heparinase** I (EC 4.2.2.7), **heparinase** II, and **heparinase** III (heparitinase; EC 4.2.2.8). **Heparinase** I degrades heparin, and **heparinase** II degrades both heparin and heparan sulfate, while **heparinase** III degrades heparan sulfate predominantly. We isolated the genes encoding **heparinases** II and III (designated hepB and hepC, respectively). These genes are not contiguous with each other or with the **heparinase** I gene (designated hepA). hepB and hepC were found to contain open reading frames of 2,316 and 1,980 bp, respectively. Enzymatic removal of pyroglutamate groups permitted sequence analysis of the amino termini of both mature proteins. It was determined that the mature forms of **heparinases** II and III contain 746 and 635 amino acids, respectively, and have calculated molecular weights of 84,545 and 73,135, respectively. The preproteins have signal sequences consisting of 26 and 25 amino acids. Truncated hepB and hepC genes were used to produce active, mature **heparinases** II and III in the cytoplasm of Escherichia coli. When these enzymes were expressed at 37degreeC, most of each recombinant enzyme was insoluble, and most of the **heparinase** III protein was degraded. When the two enzymes were expressed at 25degreeC, they were both present predominantly in a soluble, active form.

GJ2

15/3,AB/3 (Item 1 from file: 351)
DIALOG(R)File 351:DERWENT WPI
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011293799
WPI Acc No: 97-271704/199724
XRAM Acc No: C97-087314

Decreasing localised inflammatory responses - by administration of
. **heparinase** enzyme or fusion protein containing **heparinase**
enzyme and ligand which binds to activated endothelial cells

Patent Assignee: IBEX TECHNOLOGIES INC (IBEX-N)

Inventor: BENNETT D C; **CAUCHON E**; **DANAGHER P**; **FINK D**;

GROUX B; **HSIA A**; ZIMMERMAN J

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Main IPC	Week
WO 9711684	A1	19970403	WO 96US15593	A	19960927		199724 B
AU 9673791	A	19970417	AU 9673791	A	19960927		199732

Priority Applications (No Type Date): US 954622 A 19950929

Filing Details:

Patent	Kind	Filing Notes	Application	Patent
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WO 9711684 A1

Designated States (National): AU BR CA CN CZ HU IL JP KR MX NO NZ SG US
VN

Designated States (Regional): AT BE CH DE DK EA ES FI FR GB GR IE IT LU
MC NL PT SE

AU 9673791 A Based on

WO 9711684

Language, Pages: WO 9711684 (E, 74)

Abstract (Basic): WO 9711684 A

The following are claimed:

(1) decreasing localised inflammatory responses in tissues of a patient, comprising administration of **heparinase** enzyme;

(2) decreasing localised inflammatory responses in tissues of a patient, comprising administration of a fusion protein comprising a ligand which binds to activated endothelial cells and a **heparinase** enzyme;

(3) composition comprising a **heparinase** enzyme and a carrier, and

(4) composition comprising a fusion molecule comprising a ligand which binds to activated endothelium and a **heparinase** enzyme.

USE - Heparin and heparan sulphate moieties are degraded on the surface of endothelial cells and from basement membranes by administration of **heparinase**. Removal of these moieties from up-regulated proteoglycan(s) on activated endothelial cells prevents L-selectin from interacting with the proteoglycan(s). By decreasing these interactions, leukocyte rolling on activated endothelium can be inhibited. The **heparinase** may be targeted to activated endothelium by fusion of the enzymes to a binding ligand.

Dwg.0/19

15/3,AB/4 (Item 2 from file: 351)
DIALOG(R)File 351:DERWENT WPI
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008135548

WPI Acc No: 90-022549/199003

XRAM Acc No: C90-010011

Pure **heparinase**, antibodies, genes and assay methods - used in the large scale prodn. of **heparinase** for commercial and clinical applications

Patent Assignee: MASSACHUSETTS INST TECHNOLOGY (MASI)

Inventor: COONEY C L; **ZIMMERMAN J J**

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Main IPC	Week
WO 8912692	A	19891228	WO 89US2434	A	19890602	B	199003 B
EP 420894	A	19910410	EP 89907019	A	19890602	B	199115
JP 3505815	W	19911219	JP 89506606	A	19890602	B	199206
US 5169772	A	19921208	US 88203235	A	19880606	B	199252
			US 91726646	A	19910702		
EP 420894	B1	19941109	EP 89907019	A	19890602	B	199443

			WO 89US2434	A	19890602	
DE 68919360	E	19941215	DE 619360	A	19890602	B 199504
			EP 89907019	A	19890602	
			WO 89US2434	A	19890602	
EP 420894	A4	19920318	EP 89907019	A	19890000	B 199521
JP 2603349	B2	19970423	JP 89506606	A	19890602	B 199721
			WO 89US2434	A	19890602	
JP 9149788	A	19970610	JP 89506606	A	19890602	B 199733
			JP 96164991	A	19890602	

Priority Applications (No Type Date): US 88203235 A 19880606; US 91726646 A 19910702

Filing Details:

Patent	Kind	Filing Notes	Application	Patent
WO 8912692	A			
		Designated States (National): JP		
		Designated States (Regional): AT BE CH DE FR GB IT LU NL SE		
EP 420894	A			
		Designated States (Regional): AT BE CH DE FR GB IT LI LU NL SE		
US 5169772	A	Cont of	US 88203235	
EP 420894	B1	Based on	WO 8912692	
		Designated States (Regional): AT BE CH DE FR GB IT LI LU NL SE		
DE 68919360	E	Based on	EP 420894	
		Based on	WO 8912692	
JP 2603349	B2	Previous Publ.	JP 3505815	
		Based on	WO 8912692	
JP 9149788	A	Div ex	JP 89506606	

Language, Pages: WO 8912692 (E, 29); US 5169772 (8); EP 420894 (E, 10); JP 2603349 (8); JP 9149788 (10)

Abstract (Basic): WO 8912692 A

The following are claimed: (A) a method for purifying **heparinase** (I) and (I) like enzymes from Gram negative bacteria comprising: (a) disrupting the envelope of the Gram negative bacteria in an osmotically stabilised medium, e.g. 20% sucrose soln. using e.g. EDTA, lysosyme or an organic cpd., (b) releasing the non-**heparinase** like proteins from the periplasmic space of the disrupted bacteria by exposing the bacteria to a low ionic strength buffer and (c) releasing the **heparinase** like proteins by exposing the low ionic strength washed bacteria to a buffered salt soln., e.g. phosphate buffer contg. NaCl, the **heparinase** like proteins may be fractionated by polyacrylamide gel electrophoresis; (B) a purified **heparinase**-like enzyme isolated as in (A); (C) antibody to the purified (I); (D) a genetically engineered nucleic acid sequence encoding the purified (I).

(E) an assay for screening of (I)-producing bacteria comprising (a) inoculating an agar plate contg. heparin with the organism to be screened, (b) incubating the plate, (c) pouring a protamine sulphate soln. over the surface of the plate and (d) determining if a white ppte. a forms; (F) an assay for screening of (I)-producing bacteria comprising (a) providing microculture wells contg. media suitable for the organisms to be screened, (b) inoculating the wells with the organisms to be screened, (c) incubating the inoculated plates, (d) adding Azure A dye to each well, (e) measuring the absorbence of 605 nm and (f) comparing the absorbence with the absorbence of wells contg. known quantities of (I).

USE/ADVANTAGE - Highly pure (I) can be obtd. in large quantities for use in commercial and clinical applications, e.g. neutralising the anticoagulant effect of heparin in blood. The assay methods and antibodies can be used to isolate the **heparinase** genes and identify cells, e.g. host cells, producing **heparinase**.

Abstract (Equivalent): EP 420894 B

A method for purifying **heparinase** from Gram negative bacteria comprising: suspending the Gram negative bacteria in an osmotically stabilised medium; disrupting the envelope and releasing non-

heparinase proteins from the periplasmic space of the disrupted bacteria by exposing the bacteria to a buffer having the ionic strength of 10 mM phosphate and adjusted to a pH between 6.0 and 8.6; and releasing the **heparinase** from the disrupted bacteria by exposing the low ionic strength washed bacteria to a buffered salt solution having the ionic strength of a mixture of 10 mM phosphate and 0.15 M sodium chloride and adjusted to a pH between 6.0 and 8.6.

Dwg.0/1

Abstract (Equivalent): US 5169772 A

Compsn. comprising purified **heparinase** isolated from *Flavobacterium heparinum* bacteria by:- (a) disrupting the envelope without cytoplasmic proteins in an osmotically stabilised medium. (b) releasing the non-**heparinase** proteins from the periplasmic space by washing with a buffer having an ionic strength equiv. to 10mM PO₄ and of pH 6.0-8.6. (c) releasing **heparinase** by washing with a buffer of ionic strength 0.15M NaCl, pH 6.0 and 8.6. When the **heparinase** has a mol.wt. of 65-75 kD by gel chromatography and 70kKD PAGE, it is free of **heparinase** of 43KD by SDS-PAGE. USE/ADVANTAGE - **Heparinase** is produced in large, active quantities and is highly pure. Useful in commercial and clinical applications.

(Dwg.0/1

Language: ENGLISH

Document Type: CONFERENCE PAPER

Print Number: Biological Abstracts/RRM Vol. 049 Iss. 005 Ref. 075067

18/3,AB/52 (Item 7 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)
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2122852 BIOSIS Number: 63027272

HEPARINASE ACTIVITY IN LESION OF PERIODONTAL DISEASES

NAKAMURA T; SUGINAKA Y; TAKAZOE I

BULL TOKYO DENT COLL 17 (3). 1976 147-155. CODEN: BTDCA

Full Journal Title: Bulletin of Tokyo Dental College

Distribution of **heparinase**-producing Bacteroides in the lesions of periodontal diseases was investigated by comparing the materials obtained from gingival pockets and gingivectomized material from patients and dental plaque from healthy gingival crevice. Each material was cultured on the menadione supplemented blood plate, and grown mixed cells on this plate were inoculated in the heparin added Trypticase broth. Substrate determination was done by titration of a 0.02% toluidine blue solution, and through the rates of heparin-splitting by the inoculated mixed cells the distribution of the **heparinase**-producing Bacteroids was calculated. The mean rate of heparin-splitting of the materials from healthy adults was about 16.5%; that of materials from the lesions was about 70-85%, about 4-5 times as high as the former. Lesions showing high **heparinase** activity were frequently associated with strong **inflammatory** changes and many B. melaninogenicus. The presence of strains having a characteristic nature of the **heparinase**-producing Bacteroides was clearly observed in the pathological material, indicating an increase of this Bacteroides in the lesion, and its joint action with B. melaninogenicus and Propionibacterium acnes in the etiology of periodontal diseases.

18/3,AB/58 (Item 6 from file: 351)
DIALOG(R)File 351:DERWENT WPI
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010502610

WPI Acc No: 95-403932/199551

XRAM Acc No: C95-173484

Compsn. contg. 6-O-de-sulphated heparin or its fragments - has no anticoagulant activity, used for treating or preventing e.g. cancer, angiogenesis, shock, ischaemia etc.

Patent Assignee: GLYCOMED INC (GLYC-N)

Inventor: HOLME K R; ISHIHARA M; SHAKLEE P N

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Main IPC	Week
WO 9530424	A1	19951116	WO 95US5633	A	19950505	A61K-031/725	199551 B
AU 9524357	A	19951129	AU 9524357	A	19950505	A61K-031/725	199609
EP 758247	A1	19970219	EP 95918401	A	19950505	A61K-031/725	199713
			WO 95US5633	A	19950505		

Priority Applications (No Type Date): US 94239075 A 19940506

Filing Details:

Patent	Kind	Filing Notes	Application	Patent
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AU 9524357	A	Based on		WO 9530424
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EP 758247	A1	Based on		WO 9530424
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Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LI LU MC
NL PT SE

Language, Pages: WO 9530424 (E, 63); EP 758247 (E)

Abstract (Basic): WO 9530424 A

Compsn. comprises substantially unfragmented 6-O-desulphated heparin (I) or fragments of (I).

(I) has < 34 (esp. < 13)% 6-O-sulphation and up to 67 (pref. 14-28)% 2-O-sulphation.

Fragments have mol.wt. 2-6.5 (pref. about 5) kD.

USE - The compsns. are used to treat or prevent cancer, angiogenesis (e.g. diabetic retinopathy), shock (septic or hypovolaemic), ischaemia, reperfusion injury, **inflammation** and cardiovascular disease (including restenosis). (I) acts by inhibiting **heparanase**, platelet aggregation and binding of basic fibroblast growth factor to heparan sulphate.

Also, labelled (I) can be used to locate site of disease; as reagent in competitive immunoassays, and for studying pharmacokinetics.

(I) can also be used to generate specific antibodies, also useful as immunoassay reagents.

Compsns. are admin. orally, subcutaneous, intravenously or from implants, e.g. to provide 0.3 mg/kg/hr. over a period of 7-14 days.

ADVANTAGE - (I) has no significant anticoagulant activity and low toxicity. It can be produced with a controllable degree of 6-O-desulphation.

Dwg.0/8

18/3,AB/59 (Item 7 from file: 351)
DIALOG(R)File 351:DERWENT WPI
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010253796

WPI Acc No: 95-155051/199520

XRAM Acc No: C95-071406

New highly sulphated maltooligosaccharides derivs. - used to treat cancers, **inflammation**, retinopathies, cardiovascular diseases, smooth muscle cell proliferation etc.

Patent Assignee: GLYCOMED INC (GLYC-N)

Inventor: FUGEDI P; ISHIHARA M; STACK R J; TRESSLER R J; TYRRELL D J

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Main IPC	Week
WO 9509637	A1	19950413	WO 94US11368	A	19941004	A61K-031/715	199520 B
AU 9480136	A	19950501	AU 9480136	A	19941004	A61K-031/715	199532
EP 722326	A1	19960724	EP 94931314	A	19941004	A61K-031/715	199634
			WO 94US11368	A	19941004		
JP 9503510	W	19970408	WO 94US11368	A	19941004	C07H-011/00	199724
			JP 95511029	A	19941004		

Priority Applications (No Type Date): US 93133483 A 19931007

Filing Details:

Patent	Kind	Filing Notes	Application	Patent
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WO 9509637 A1

Designated States (National): AU CA JP

Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE

AU 9480136 A Based on WO 9509637

EP 722326 A1 Based on WO 9509637

Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

JP 9503510 W Based on WO 9509637

Language, Pages: WO 9509637 (E, 44); EP 722326 (E); JP 9503510 (42)

Abstract (Basic): WO 9509637 A

Highly sulphated maltooligosaccharide cpds. of formula (I) are new: X = O or S; R1 = alkyl, aryl, aralkyl, a reduced or oxidised glucose unit, SO3M or H; R2 = SO3M or H; M = biologically acceptable cation; n = 1-9; provided that at least 50% of R2 gps. are sulphated.

USE - Cpds. (I) have heparin like properties. (I) can be used to treat or prevent a variety of diseases including cancer pref. metastatic cancer, cardiovascular diseases, **inflammation** and diseases caused or exacerbated by platelet aggregation or angiogenesis

e.g. retinopathies and cancers and to treat diseases caused by excessive and destructive smooth muscle cell proliferation. (I) can also treat diseases of viral origin. Metastatic cancer is treated by (I) by inhibiting **heparanase**. Angiogenesis is associated with certain diseases e.g. arthritis, retinopathies esp. diabetic retinopathy and tumours. The smooth muscle proliferation may be due to traumas such as in cases of surgical patients where wounds or surgery results in vascular damage a 2 deg. smooth muscle cell proliferation. As in addition to trauma certain diseases are associated with unwanted vascular proliferation e.g. Goodpasture syndrome, acute glomerulonephritis, neonatal pulmonary hypertension, asthma, congestive heart failure, adult pulmonary hypertension and renal vascular hypertension.

Dwg.0/0

melief

Set	Items	Description
S1	2338	HEPARINASE?
S2	372209	INFLAMMATORY
S3	0	L2 ND L1
S4	42	S1 AND S2
S5	21	RD (unique items)
S6	6298	MIP
S7	459	L6 AND L1
S8	0	L6 (P) L1
S9	7	S6 AND S1
S10	3	RD (unique items)
S11	81237	TNF
S12	11	S11 AND S1
S13	4	RD (unique items)
S14	1173	IL-1
S15	0	S14 AND S1
S16	4063	RANTES
S17	7	S16 AND S1
S18	3	RD (unique items)
S19	54250	IL(W)1
S20	5	S19 AND S1
S21	2	RD (unique items)
S22	5	S21 OR S18
S23	5	RD (unique items)
S24	30891	IL(W)4
S25	1	S24 AND S1



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Bib Data Sheet

CONFIRMATION NO. 4359

SERIAL NUMBER 08/722,659	FILING DATE 09/27/1996 RULE	CLASS 435	GROUP ART UNIT 1644	ATTORNEY DOCKET NO. 104385.140
APPLICANTS D. CLARK BENNETT, MONTREAL, CANADA; ELIZABETH CAUCHON, MONTREAL, CANADA; DOMINIQUE FINK, MONTREAL, CANADA; BRIGETTE GROUX, MONTREAL, CANADA; ARIANE HSIA, MONTREAL, CANADA; PAMELA DANAGHER, MONTREAL, CANADA; JOSEPH ZIMMERMANN, MONTREAL, CANADA;				
** CONTINUING DATA *****				
** FOREIGN APPLICATIONS *****				
** SMALL ENTITY **				
Foreign Priority claimed <input type="checkbox"/> yes <input type="checkbox"/> no 35 USC 119 (a-d) conditions met <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> Met after Allowance Verified and Acknowledged _____ Examiner's Signature _____ Initials _____		STATE OR COUNTRY CANADA	SHEETS DRAWING 19	TOTAL CLAIMS 17
		INDEPENDENT CLAIMS 6		
ADDRESS HOLLIE L. BAKER HALE & DORR LLP. 60 STATE STREET BOSTON ,MA 02109				
TITLE USE OF HEPARINASE TO DECREASE INFLAMMATORY RESPONSES				
FILING FEE RECEIVED 700	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:		<input type="checkbox"/> All Fees <input type="checkbox"/> 1.16 Fees (Filing) <input type="checkbox"/> 1.17 Fees (Processing Ext. of time) <input type="checkbox"/> 1.18 Fees (Issue) <input type="checkbox"/> Other _____ <input type="checkbox"/> Credit	

APPLICATION NUMBER 08/722,659	FILING DATE 09/27/1996	CLASS 435	GROUP ART UNIT 1644	ATTORNEY DOCKET NO 104385.140
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APPLICANT
D. CLARK BENNETT, MONTREAL, CANADA; ELIZABETH CAUCHON, MONTREAL, CANADA;
DOMINIQUE FINK, MONTREAL, CANADA; BRIGETTE GROUX, MONTREAL, CANADA;
ARIANE HSIA, MONTREAL, CANADA; PAMELA DANAGHER, MONTREAL, CANADA; JOSEPH
ZIMMERMANN, MONTREAL, CANADA.

CONTINUING DOMESTIC DATA***
VERIFIED

371 (NAT'L STAGE) DATA***
VERIFIED

FOREIGN APPLICATIONS***
VERIFIED

*allowance due to TD.
all rejections based on
1863 patent.*

SMALL ENTITY

Foreign priority claimed 35 USC 119 (a-d) conditions met	<input type="radio"/> yes <input type="radio"/> no <input type="radio"/> yes <input type="radio"/> no <input type="radio"/> Met after Allowance	STATE OR COUNTRY CAX	SHEETS DRAWINGS 19	TOTAL CLAIMS 17	INDEPENDENT CLAIMS 6
Verified and acknowledged _____ Examiner's Name Initials					

ADDRESS
HOLLIE L. BAKER
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TITLE
USE OF HEPARINASE TO DECREASE INFLAMMATORY RESPONSES

FILING FEE RECEIVED \$**570	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for the following:	<input type="radio"/> All Fees <input type="radio"/> 1.16 Fees (Filing) <input type="radio"/> 1.17 Fees (Processing Ext. of Time) <input type="radio"/> 1.18 Fees (Issue) <input type="radio"/> Other _____ <input type="radio"/> Credit
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